



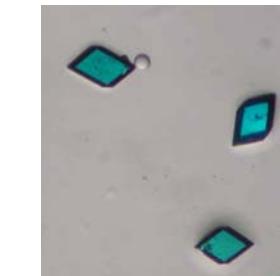
Single Crystal Diffraction

Cheiron School, Spring8, 2012

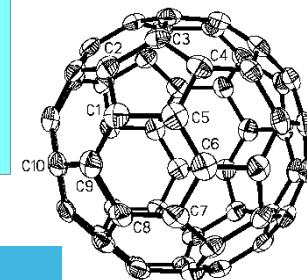
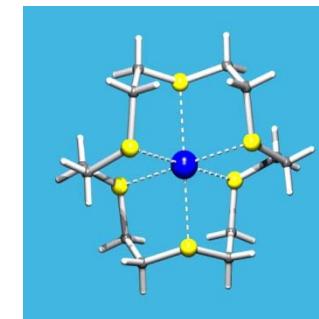
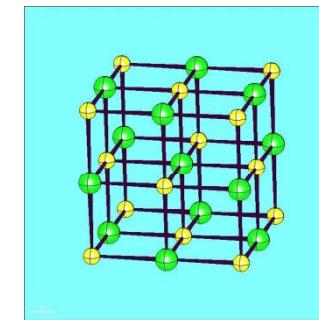
Claire Wilson



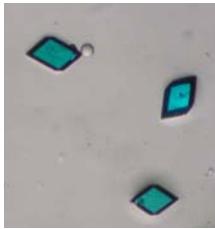
Our aim



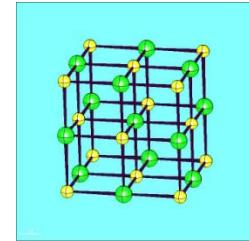
Xray diffraction



- To explore getting from a crystal to a structural model



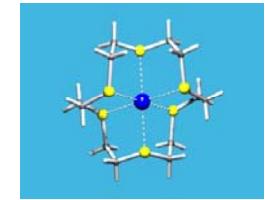
Single Crystals



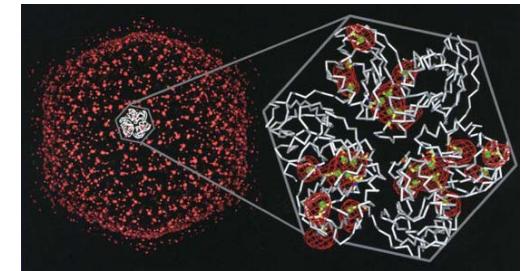
Material in solid state with 3-d translational symmetry

- Crystals may be:

- elements (e.g. diamond), minerals, inorganic salts, molecular materials (organic, metal complexes), extended lattice structures (MOFs), macromolecules (proteins, DNA, viruses)

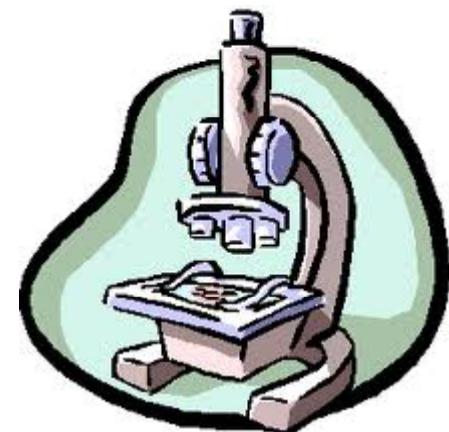
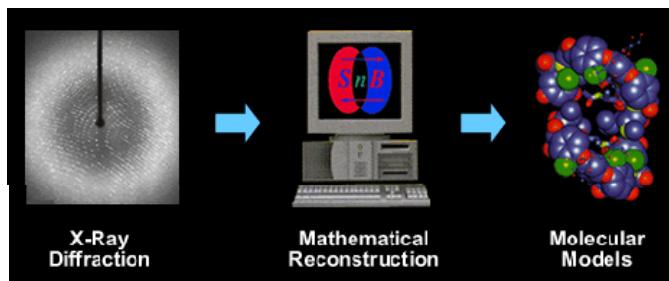


For synchrotron single crystal diffraction experiments crystals are typically 10 – 100 microns in size ($1\mu\text{m} = 1 \times 10^{-6}\text{m} = 10,000\text{\AA}$)

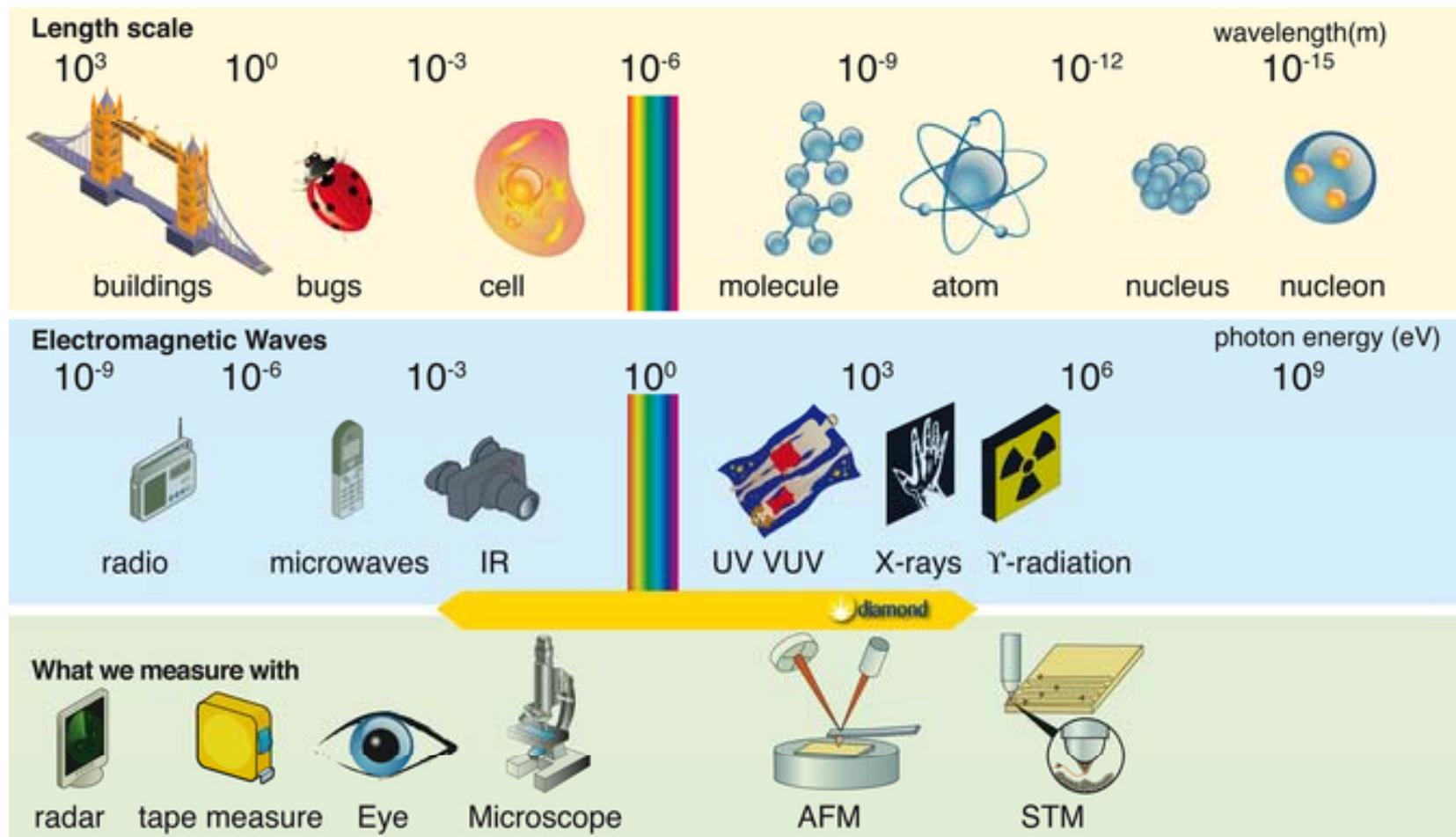


Microscope analogy

- With visible light look at objects similar size to wavelength (400-700nm)
- For atoms and molecules radiation with wavelength of similar size is xrays
- Reform image mathematically and not in real time

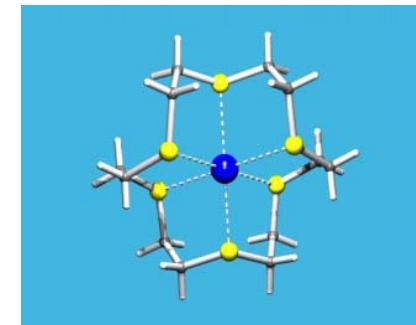
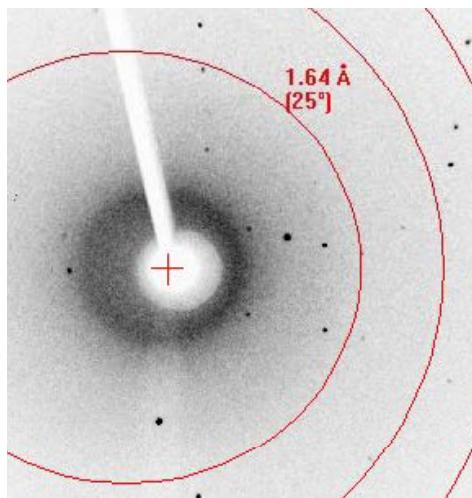


The many **colours** of light



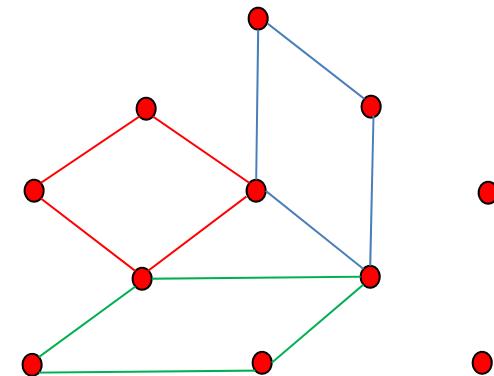
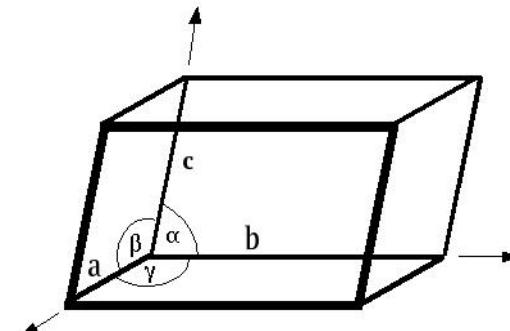
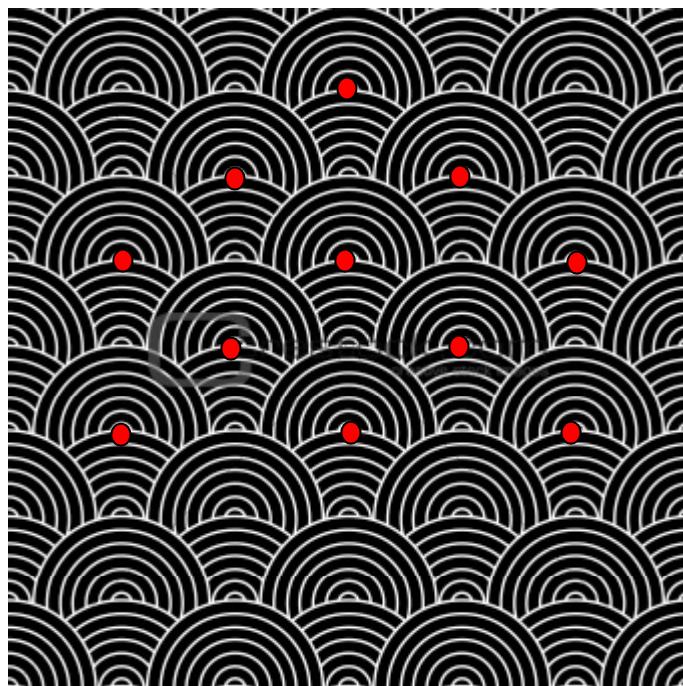
Why do we need a crystal?

- Identical repeat unit repeated infinitely in 3 dimensions leads to diffraction
- Measure position and intensity of diffracted beams and work back to the structure



Lattice

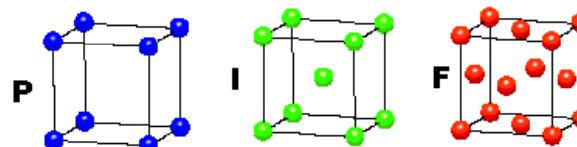
- Array of points equivalent by translation – lattice
- Unit cell describes geometry of lattice



Crystal Systems

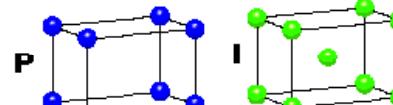
CUBIC

$a = b = c$
 $\alpha = \beta = \gamma = 90^\circ$



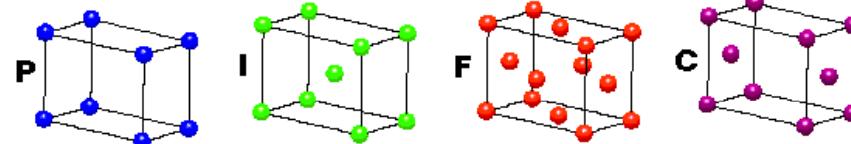
TETRAGONAL

$a = b \neq c$
 $\alpha = \beta = \gamma = 90^\circ$



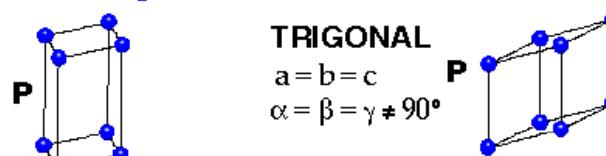
ORTHORHOMBIC

$a \neq b \neq c$
 $\alpha = \beta = \gamma = 90^\circ$



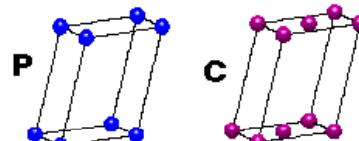
HEXAGONAL

$a = b \neq c$
 $\alpha = \beta = 90^\circ$
 $\gamma = 120^\circ$



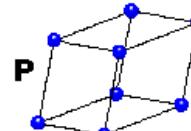
MONOCLINIC

$a \neq b \neq c$
 $\alpha = \gamma = 90^\circ$
 $\beta \neq 120^\circ$



TRICLINIC

$a \neq b \neq c$
 $\alpha \neq \beta \neq \gamma \neq 90^\circ$



4 Types of Unit Cell

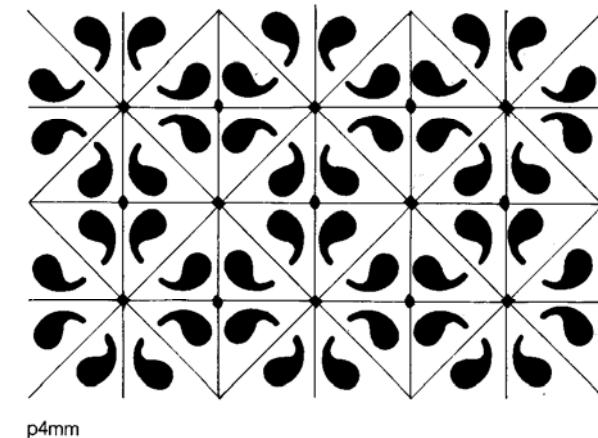
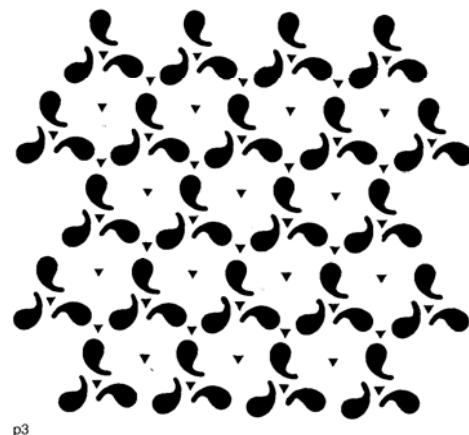
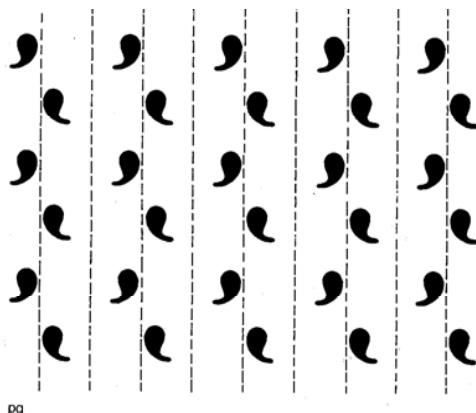
P = Primitive
I = Body-Centred
F = Face-Centred
C = Side-Centred

+
7 Crystal Classes
→ 14 Bravais Lattices

Symmetry

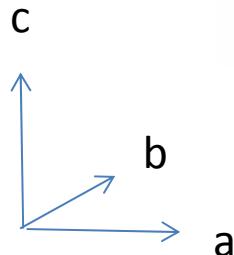
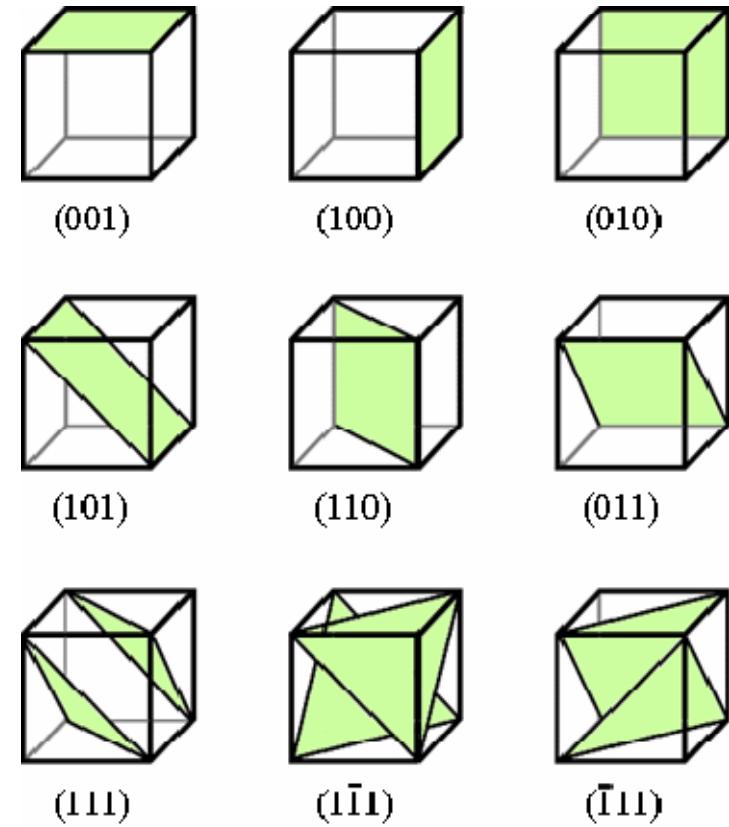
Definition

- A **crystallographic symmetry operation** is a symmetry operation, which maps a (periodic) crystal structure onto itself.

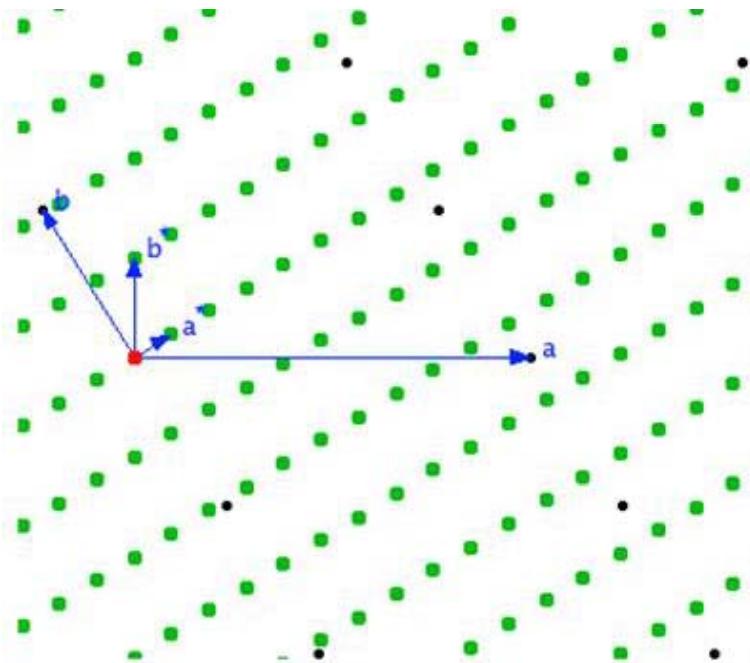


Miller indices hkl

- Description of orientation of planes slicing through crystal



Reciprocal lattice



- Valuable construct in diffraction
- Reciprocal geometric relationship between crystal lattice and its diffraction pattern

<http://escher.epfl.ch/rllattice/>

Reciprocal lattice relationships

- $\mathbf{a}^* \cdot \mathbf{b} = \mathbf{a}^* \cdot \mathbf{c} = \mathbf{b}^* \cdot \mathbf{a} = \mathbf{b}^* \cdot \mathbf{c} = \mathbf{c}^* \cdot \mathbf{a} = \mathbf{c}^* \cdot \mathbf{b} = 0$
- $\mathbf{a}^* \cdot \mathbf{a} = \mathbf{b}^* \cdot \mathbf{b} = \mathbf{c}^* \cdot \mathbf{c} = 1$
- $\mathbf{a}^* = (\mathbf{b} \times \mathbf{c}) / V(4)$
- $\mathbf{b}^* = (\mathbf{c} \times \mathbf{a}) / V(5)$
- $\mathbf{c}^* = (\mathbf{a} \times \mathbf{b}) / V(6)$
- $V^* = \mathbf{a} \cdot \mathbf{b} \times \mathbf{c}$

Reciprocal lattice vector

$$\mathbf{h} = h\mathbf{a}^* + k\mathbf{b}^* + l\mathbf{c}^*$$

$$\mathbf{a} \times \mathbf{b} = |\mathbf{a}| |\mathbf{b}| \sin \gamma \quad \mathbf{a} \cdot \mathbf{b} = |\mathbf{a}| |\mathbf{b}| \cos \gamma$$

Diffraction

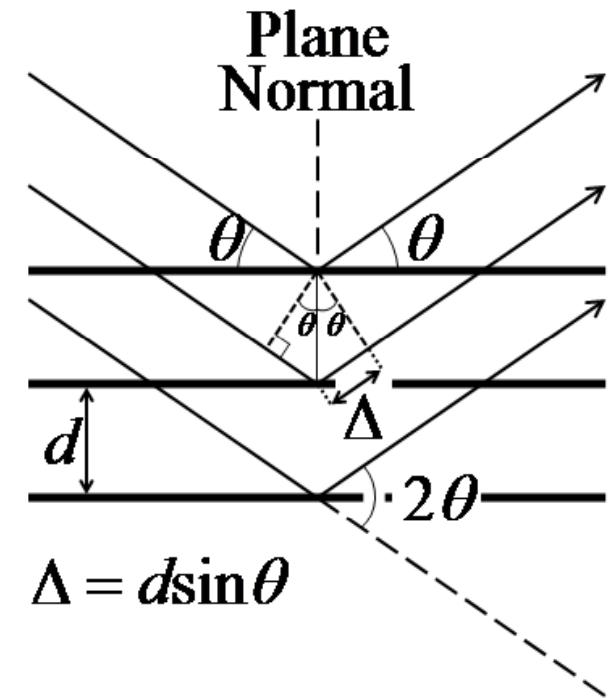
- Radiation scattered in all directions
- only measurable intensity where constructive interference – scattered waves are in phase
- x-rays scattered by different points must have path length differences equal to $n\lambda$
- Laue Conditions – 3 conditions in 3D must be simultaneously satisfied to have diffraction

The Bragg Equation

- Consider diffraction as if reflection from sets of planes passing through lattice points
- Path length difference of the scattered rays from adjacent planes is 2Δ

$$n\lambda = 2d_{hkl} \sin\theta$$

Usually ignore n and consider n=2 as scattering from planes 2h,2k,2l



Scattering vector

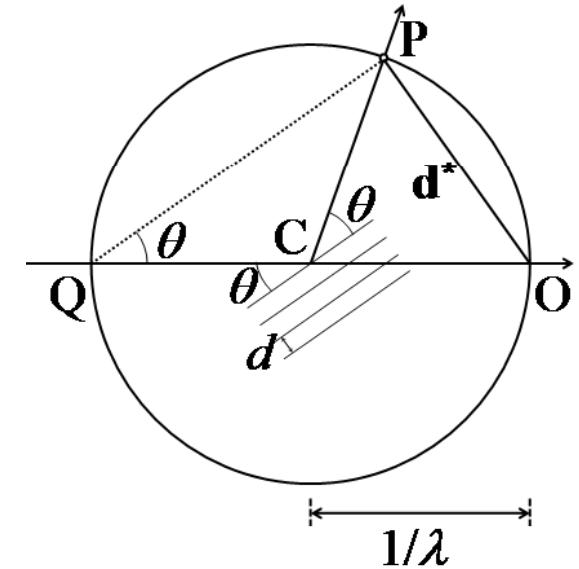
- In plane of and bisecting the incident and diffracted beams with magnitude $1/d$ or d^*
- Rearrange Bragg equation

$$\sin \theta = (\lambda/2) (1/d)$$

scattering angle related to $1/d$

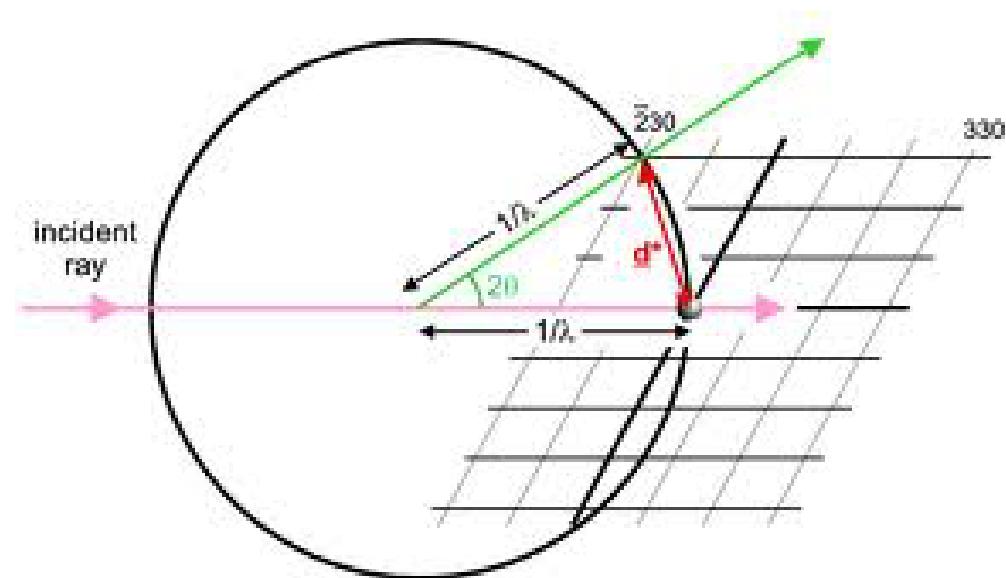
Ewald sphere

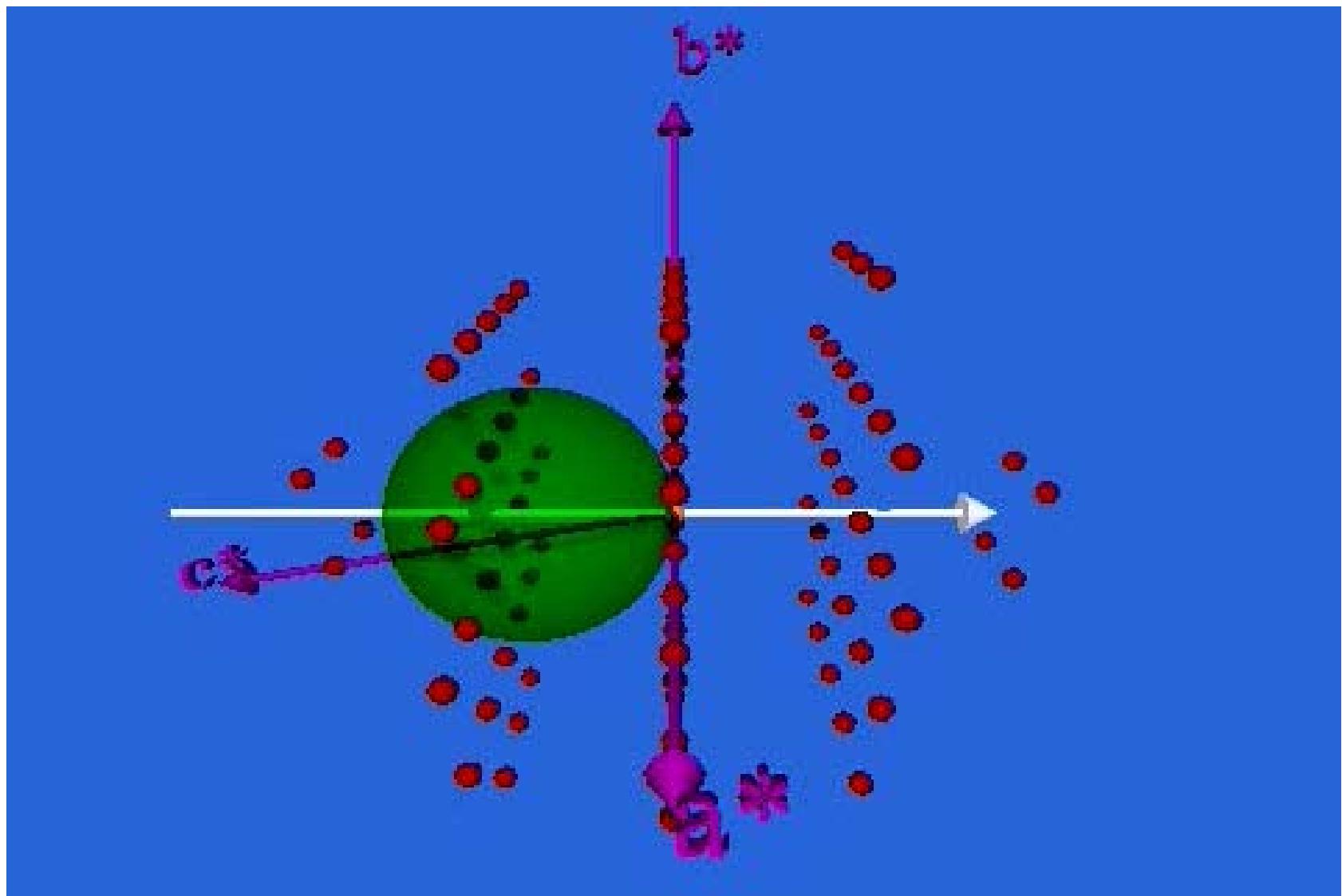
- For monochromatic beam - sphere radius $1/\lambda$ centered on crystal (C)
- Incident beam enters at Q
- Origin of reciprocal space at O
- Bragg equation is satisfied when node P on surface of sphere the $\sin\theta = OP/QO = d^*/(2/\lambda) = \lambda/2d$



Ewald sphere

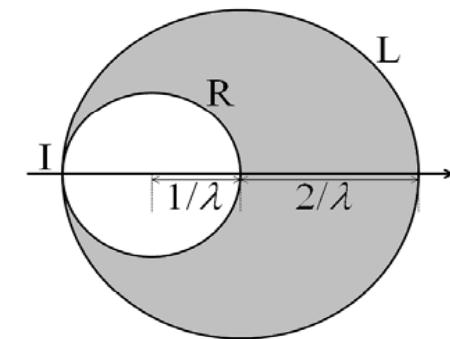
- Crystal rotated to bring reciprocal lattice points into diffracting position
- Multiple diffraction
- Lorentz Factor
- Blind regions





Limiting Sphere

- Boundary between observable and unobservable reflections for a given wavelength, λ
- Long wavelengths – small limiting sphere – lower resolution data obtainable



L is the limiting sphere
radius $2/\lambda$,
 R the reflecting sphere
radius $1/\lambda$

Intensity of Diffracted beams

- Lattices and unit cells describe geometry of diffraction
- Diffracted intensities differ for different reflections – depend on orientation of plane in crystal
- Weak and strong intensities – both contain information
- Weaker at high scattering angles – atomic scattering factor and temperature factor
- Depend on unit cell contents – atom types and positions
- $I(\mathbf{h}) = k |F(\mathbf{h})|^2$

Structure factor $F(\mathbf{h})$

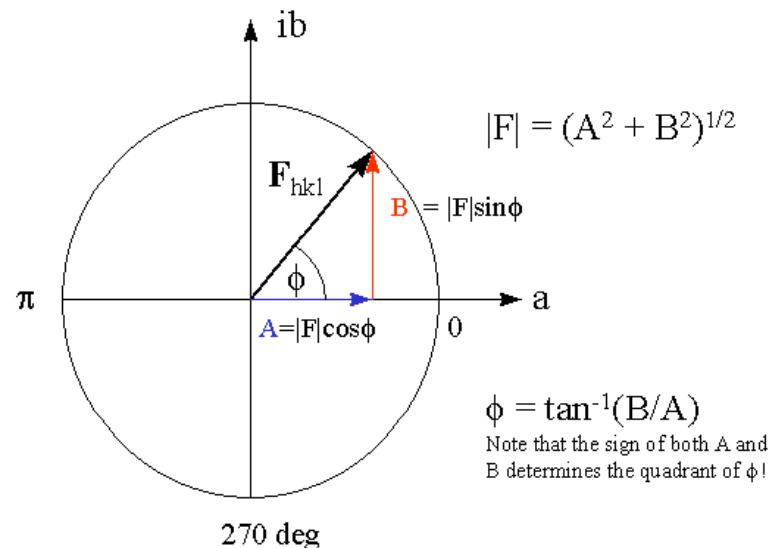
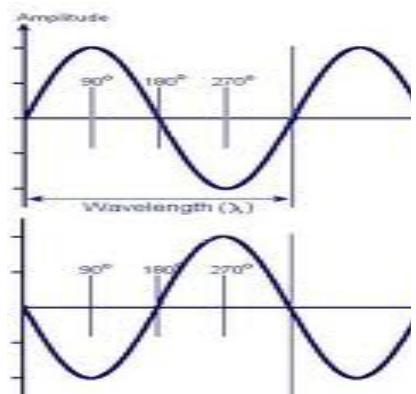
$$F(\mathbf{h}) = \sum_{j=1}^n f_j e^{2\pi i \mathbf{h} \cdot \mathbf{x}_j} = |F(\mathbf{h})| e^{i\phi(\mathbf{h})}$$

- Structure factor for a given plane or reflection (\mathbf{h})
- Summed over all j atoms in unit cell, with positions \mathbf{x}_j and that have scattering power f_j – atomic scattering factor
- $\mathbf{x}_j = x_j \mathbf{a} + y_j \mathbf{b} + z_j \mathbf{c}$ position of atom j
- $\mathbf{h} = h \mathbf{a}^* + k \mathbf{b}^* + l \mathbf{c}^*$
- $\mathbf{h} \cdot \mathbf{x}_j = h x_j + k y_j + l z_j$

Structure factor representation

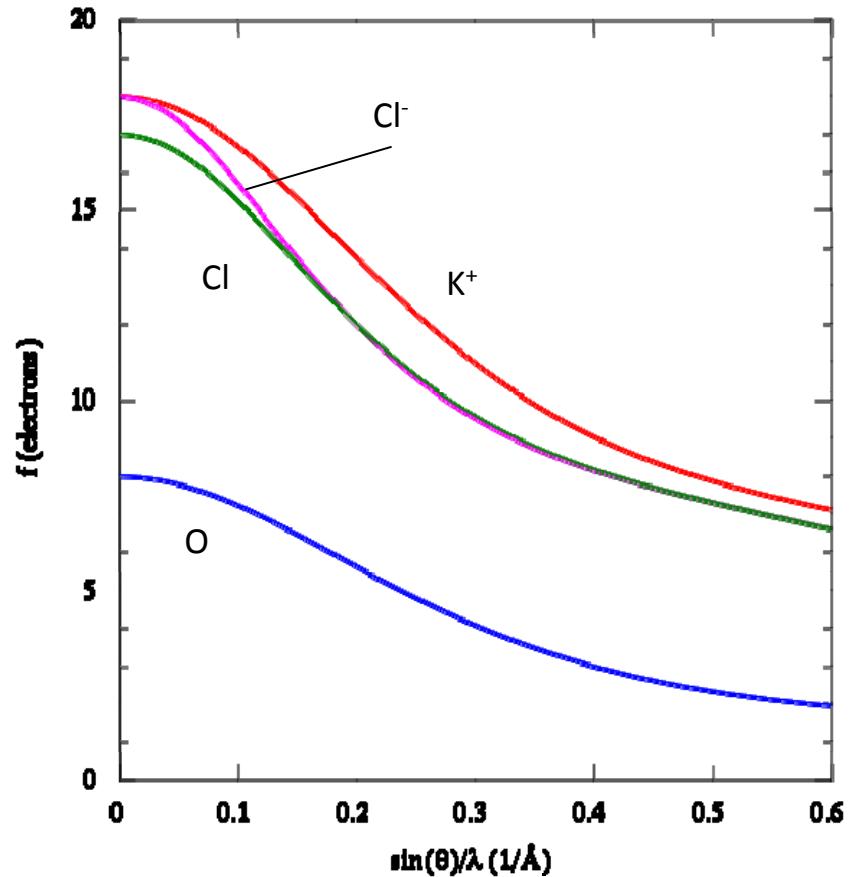
$$F(\mathbf{h}) = |F(\mathbf{h})| e^{i\phi(\mathbf{h})} = A(\mathbf{h}) + iB(\mathbf{h}) = \\ |F(\mathbf{h})|(\cos \phi(\mathbf{h}) + i \sin \phi(\mathbf{h}))$$

- Each reflection (diffracted wave) has amplitude $|F|$ and phase ϕ

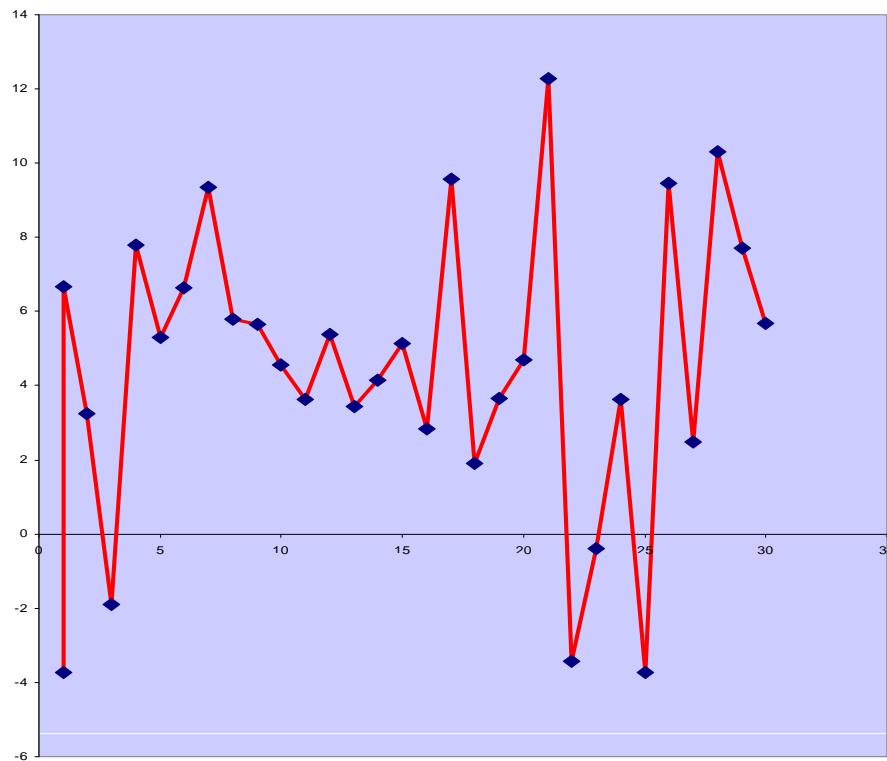


Xray atomic scattering factors

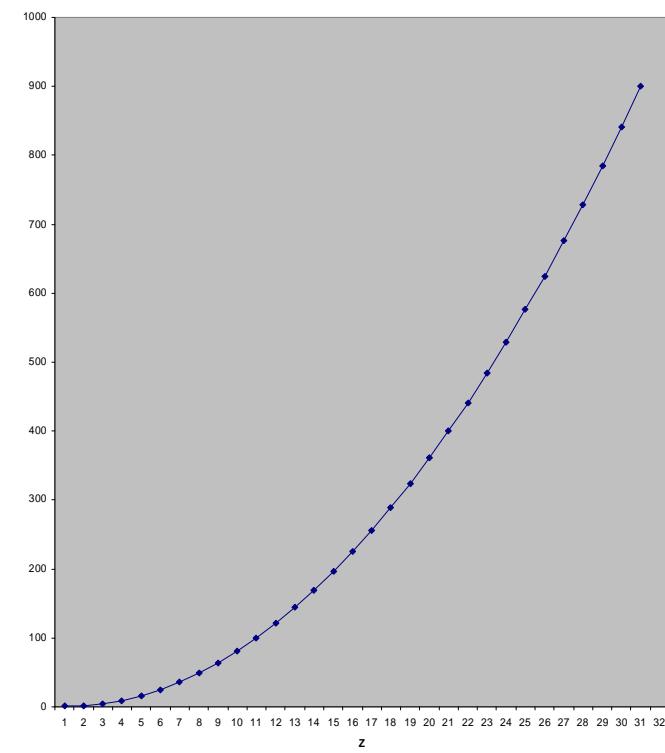
- Not point scatterer – electron cloud
- $2\theta=0^\circ$ electrons scatter in phase = atomic number



Neutron scattering



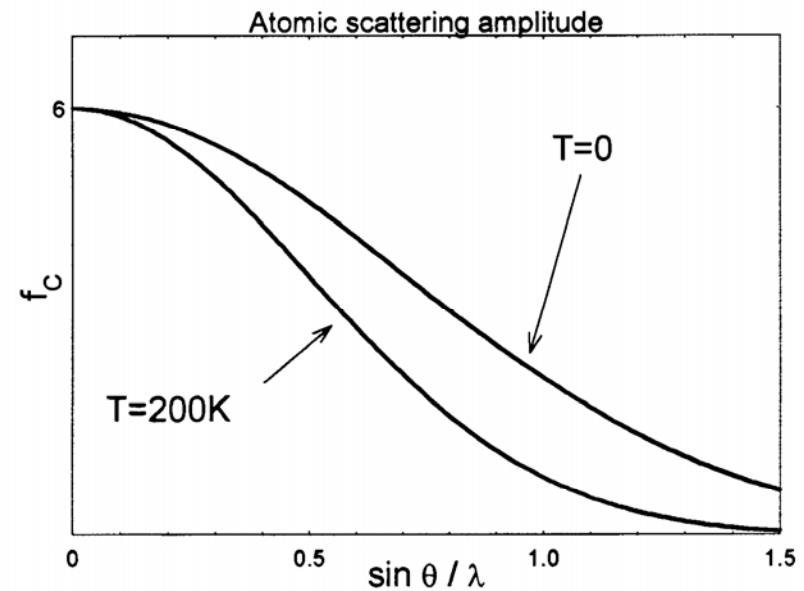
Neutron scattering length (fm)
– random variation with Z



scattered intensity for X-rays proportional to Z^2

Atomic scattering factors and atomic displacements

- Atoms in crystals not stationary but vibrating
- Intensity reduced at higher T and higher angles by temperature or Debye-Waller factor, B
- U, mean displacement of atom



$$f_B = f \cdot e^{-B(\sin \theta / \lambda)^2}$$

$$B = 8\pi^2 \langle u^2 \rangle$$

Structure factor

$$F(\mathbf{h}) = \int_{\text{cell}} \rho(\mathbf{x}) d\mathbf{x} e^{2\pi i \mathbf{h}\cdot\mathbf{x}} = \sum_{j=1}^n f_j e^{2\pi i \mathbf{h}\cdot\mathbf{x}_j}$$

- $F(\mathbf{h})$ integrated electron density ρ at every point \mathbf{x} in the unit cell
- Or summation atomic scattering factors f_j (all j atoms in unit cell)
- Structure factor (phase and amplitude) - Fourier transform of the electron density

Electron density

$$\rho(\mathbf{x}) = \frac{1}{V} \sum_{\mathbf{h}} F(\mathbf{h}) e^{-2\pi i \mathbf{h} \cdot \mathbf{x}}$$

V = unit cell volume

- $\rho(\mathbf{x})$ inverse Fourier transform of $F(\mathbf{h})$
- With all $F(\mathbf{h})$ (phase and amplitude) - complete description of unit cell contents

The ‘Phase problem’

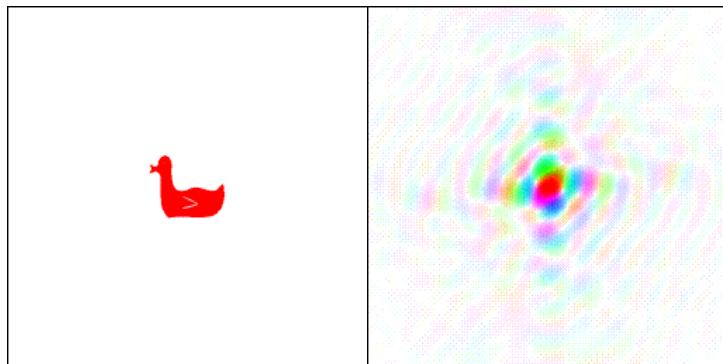
$$\rho(x) = \frac{1}{V} \sum_{\mathbf{h}} F(\mathbf{h}) e^{-2\pi i \mathbf{h} \cdot \mathbf{x}}$$

But do not have all information to reconstruct $\rho(x)$

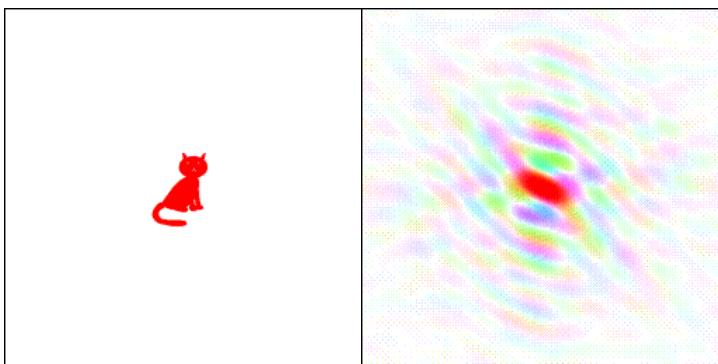
$$F(\mathbf{h}) = \sum_{j=1}^n f_j e^{2\pi i \mathbf{h} \cdot \mathbf{x}_j} = |F(\mathbf{h})| e^{i\phi(\mathbf{h})}$$

- ✓ V - unit cell volume
- ✓ $|F(\mathbf{h})|$ - structure factor amplitude (proportional to measured intensities)
- ✗ $\phi(\mathbf{h})$ – phase information – don’t measure

Is the phase important?



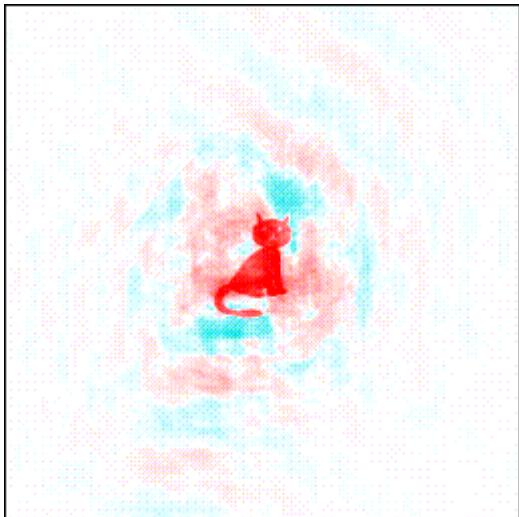
A duck and its Fourier transform



and a cat and its Fourier transform

From Kevin Cowtan's Book of Fourier
<http://www.ysbl.york.ac.uk/~cowtan/fourier/magic.html>

Is the phase important?



- Take amplitudes (magnitudes) from duck transform with the phases from the cat transform and when you reconstruct an image it is recognisable as a cat
- The image that contributed to the magnitudes has gone

From Kevin Cowtan's Book of Fourier

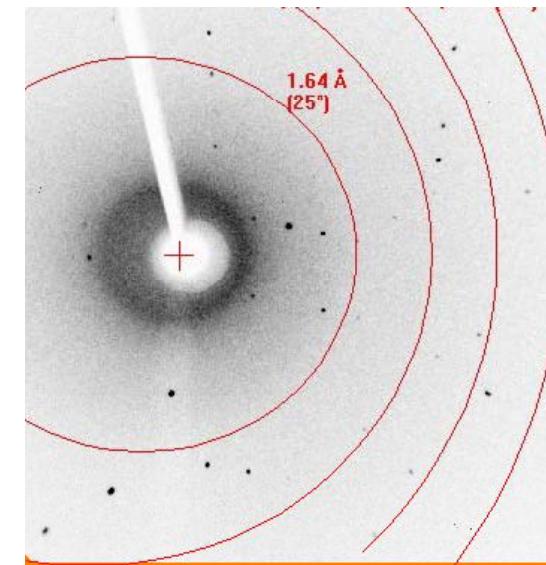
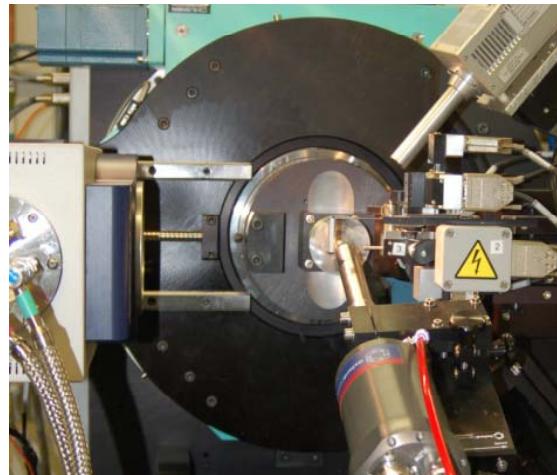
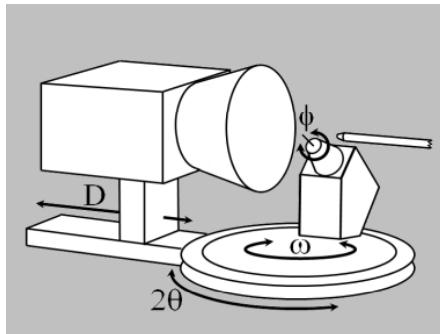
<http://www.ysbl.york.ac.uk/~cowtan/fourier/magic.html>

Is the phase important?

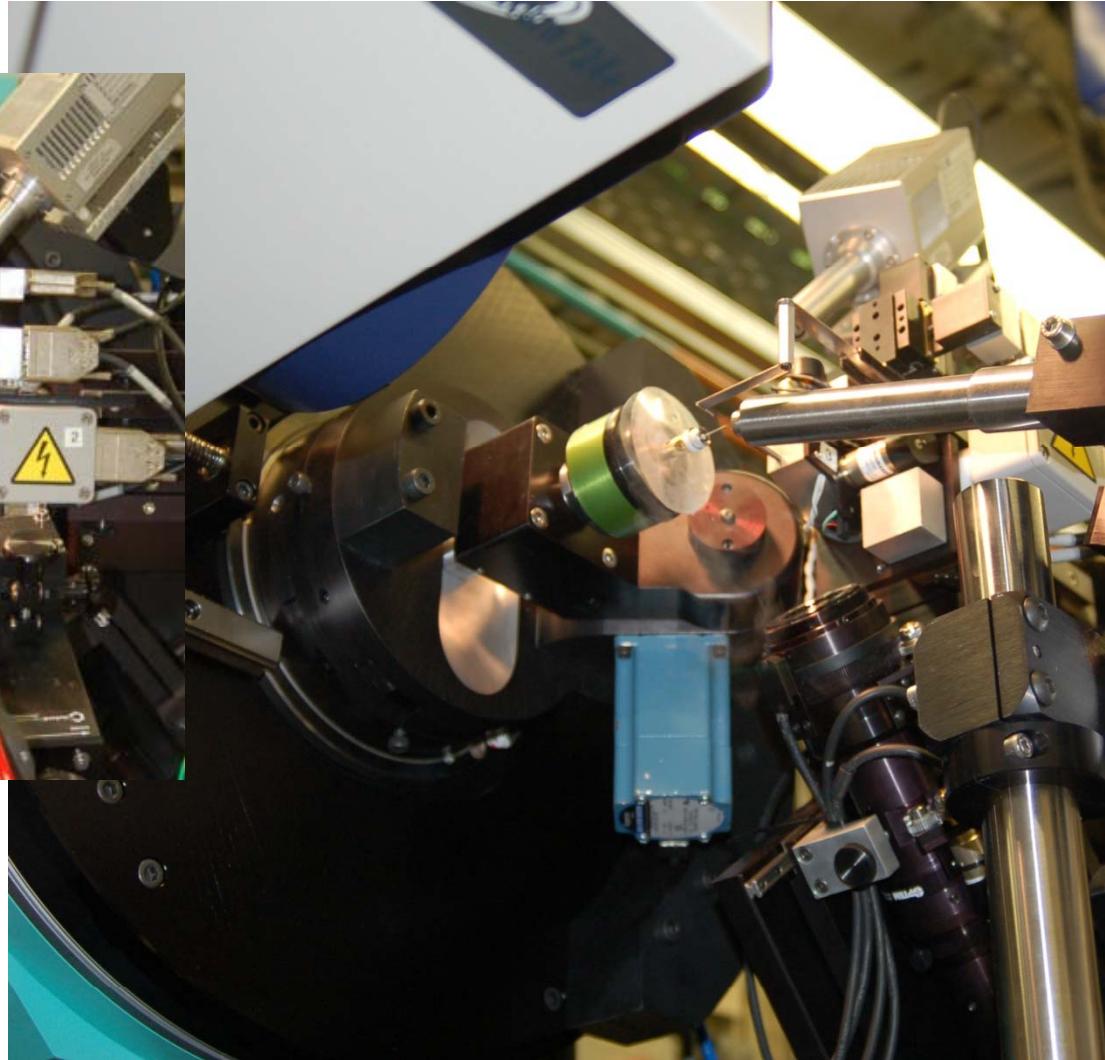
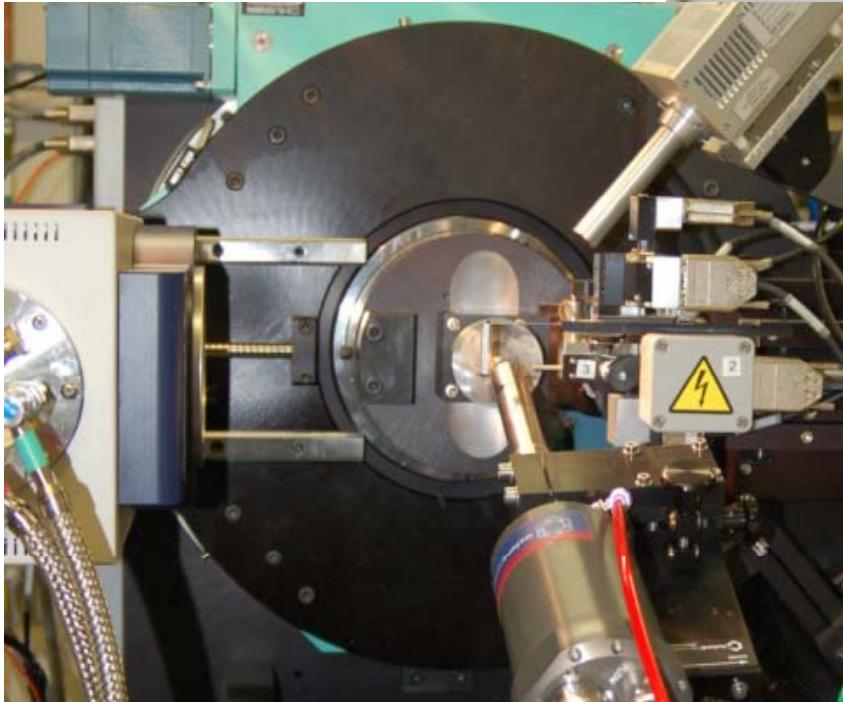
- The phase contains much more information about the atomic positions than the amplitudes
- Need some way to obtain estimated values for the phase and to ‘solve’ the structure

Data collection

- Xray source
- Diffractometer – circles to move crystal
- Detectors - CCD, image plate, pixel detectors to measure position and intensity of diffracted intensities



Diffractometer



<http://phillips-lab.biochem.wisc.edu/xrayviewuse.html>

Experimental considerations

- Some examples of experimental choices:
 - choice of crystal
 - wavelength to use
 - sample environment e.g. temperature
 - exposure time
 - resolution of data
- Depending on material and nature of study

Data reduction

- Convert measured intensities to $|F_{\text{obs}}|$ - ‘observed structure factor amplitudes’ with associated standard uncertainty, $\sigma(F_{\text{obs}})$
- Corrections applied e.g.
 - Lorenz-polarisation effects
 - Absorption correction – absorption by crystal depends on path length through crystal for a given reflection, unit cell contents, wavelength
- Unit cell parameters
- Space group determination
- Result – list of reflections as $h, k, l, |F_{\text{obs}}|, \sigma(F_{\text{obs}})$

Structure Solution methods

- Direct methods
- Heavy-atom (Patterson) methods
- Charge flipping
 - Fragment searches
 - Inference
 - isostructural relationships

Result may be one heavy atom site or complete non-hydrogen structure depending on method and success level

Model development

$$F(\mathbf{h}) = \sum_{j=1}^n f_j e^{2\pi i \mathbf{h} \cdot \mathbf{x}_j} = |F(\mathbf{h})| e^{i\phi(\mathbf{h})}$$

- Calculate amplitudes $|F_{\text{calc}}(\mathbf{h})|$ and phases as now ‘know’ some atom positions \mathbf{x}_j from trial structure
- Fourier transform $|F_{\text{obs}}(\mathbf{h})|$ with *calculated* phases – **new** information - model density plus additional atoms not included in model
- Difference Fourier maps using $|F_{\text{obs}}(\mathbf{h})| - |F_{\text{calc}}(\mathbf{h})|$ missing atoms/misassigned atoms clearer

Model development and structure refinement

- Include ‘new’ atoms in model and repeat until all atoms located
- Model improvements – displacement parameter modeling – isotropic-anisotropic, disorder, hydrogen atoms
- Least squares refinement - minimise $|F_{\text{obs}}(\mathbf{h})| - |F_{\text{calc}}(\mathbf{h})|$ to give best fit of model

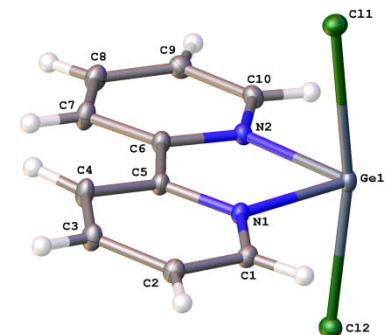
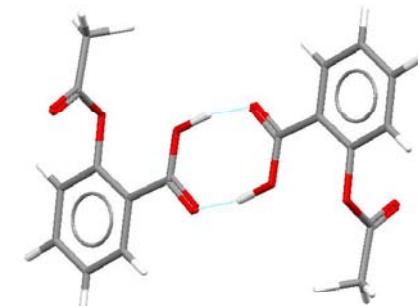
Structure model

Typically consists of

- Atom type e.g. C, N, Fe (which atomic form factor to use)
- Atom positions in fractional coordinates x,y,z
- Site occupancy
- Atomic displacement parameters – usually isotropic Uiso or anisotropic Uij
- Unit cell and space group

Results and interpretation

- Result - electron density map
- Refined model
 - coordinates of atoms, x,y,z with estimated error, at centre of electron density peaks
 - Atomic displacement parameters
 - Unit cell parameters and space group
- Interpret - bond lengths and angles for ‘bonded’ atoms, torsion angles
- Intermolecular distances e.g. H-bonding.



Web resources

- Kevin Cowtan
<http://www.ysbl.york.ac.uk/~cowtan/>
- Gervais Chapuis
<http://escher.epfl.ch/eCrystallography/>
- Xrayview <http://phillips-lab.biochem.wisc.edu/xrayviewuse.html>
- Joe Reibenspies
<http://xray.tamu.edu/courses.php>